Figure 1

A

CIRS	X	CIRS
BS'		BB'

donor molecule with donor fragment

IRS	Y	IRS
PP'		PP'

receptor molecule with receptor fragment

IRS recombinase
e.g. phage φC31 Int protein

	Y	
BP'		PB'

donor molecule with receptor fragment

	X	
PB'		BP'

receptor molecule with donor fragment

B

RRS-1	X	RRS-2

donor molecule with donor fragment

RRS-1	Y	RRS-2

receptor molecule with receptor fragment

RRS recombinase
e.g. phage P1 Cre protein

RRS-1	Y	RRS-2

donor molecule with receptor fragment

RRS-1	X	RRS-2

receptor molecule with donor fragment

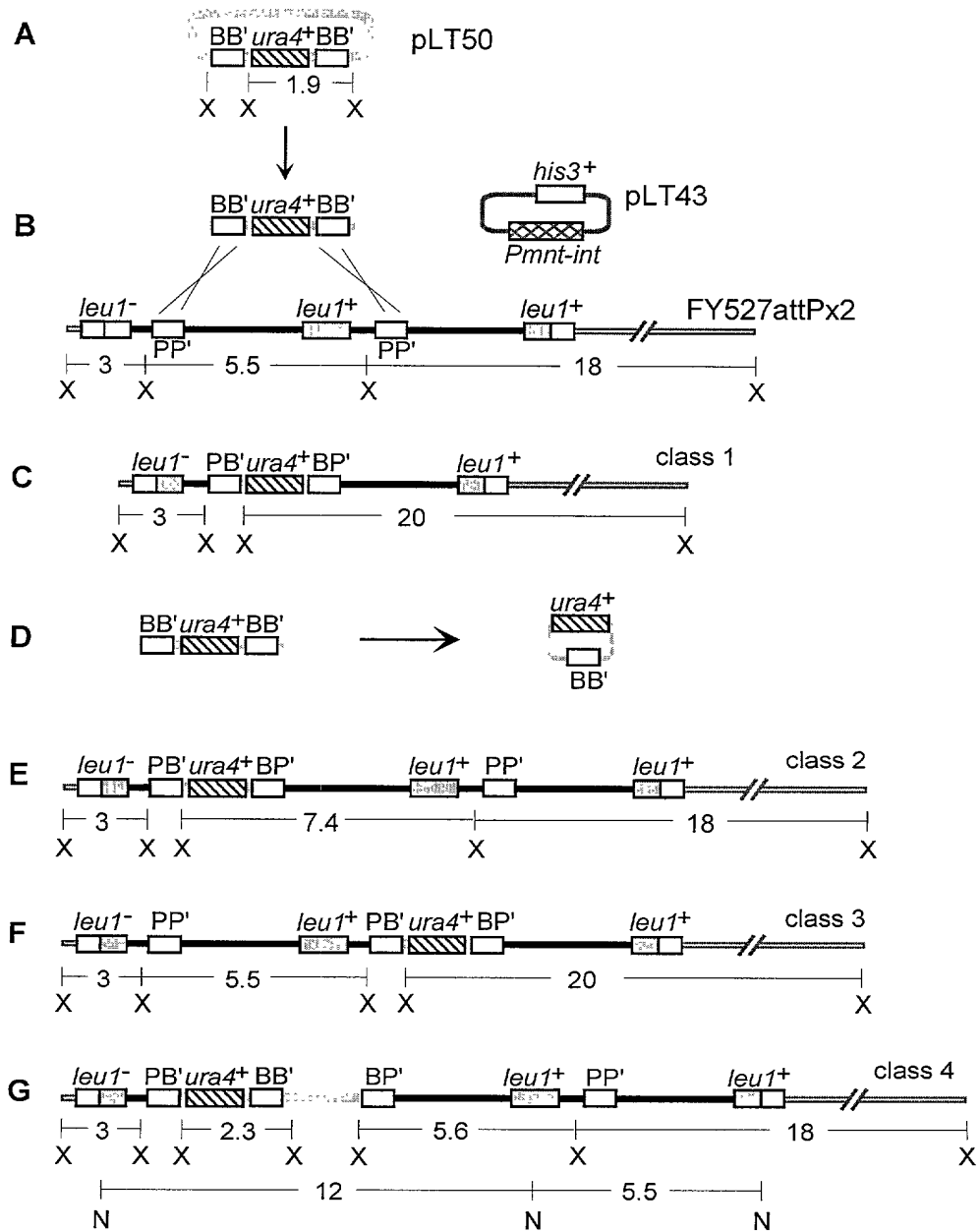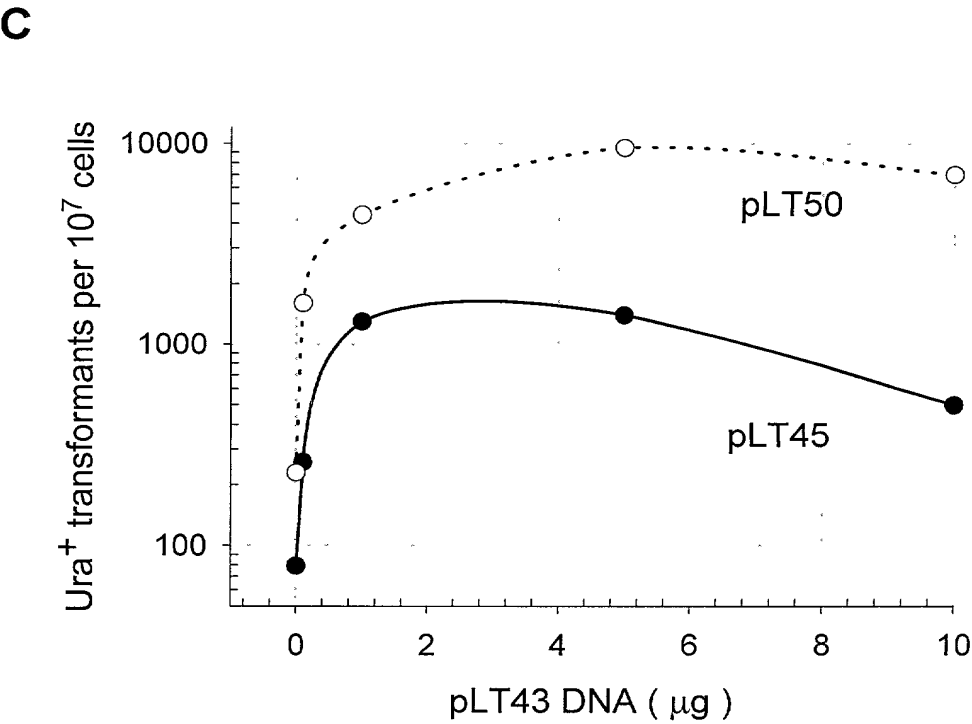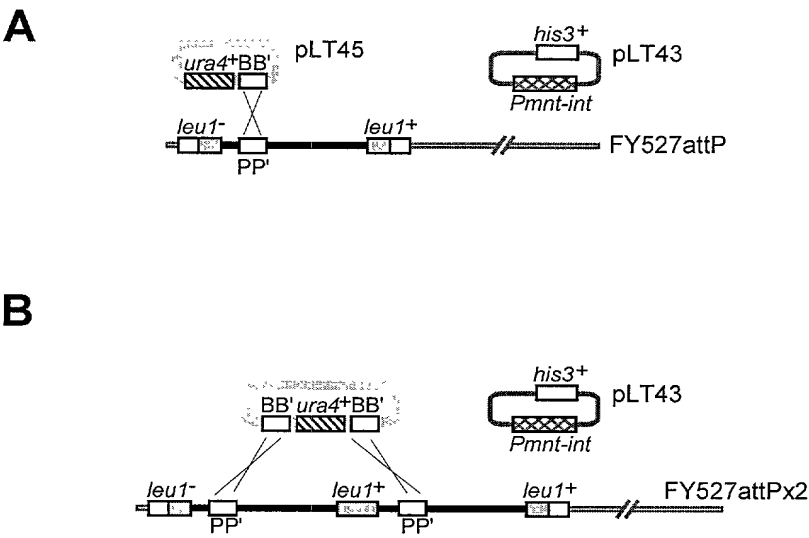|	
PP'	= *attP*
BB'	= *attB*
PB'	= *attR*
BP'	= *attL*
◄	= *loxP*
◁	= *lox511*

Figure 2

Figure 3

Figure 4

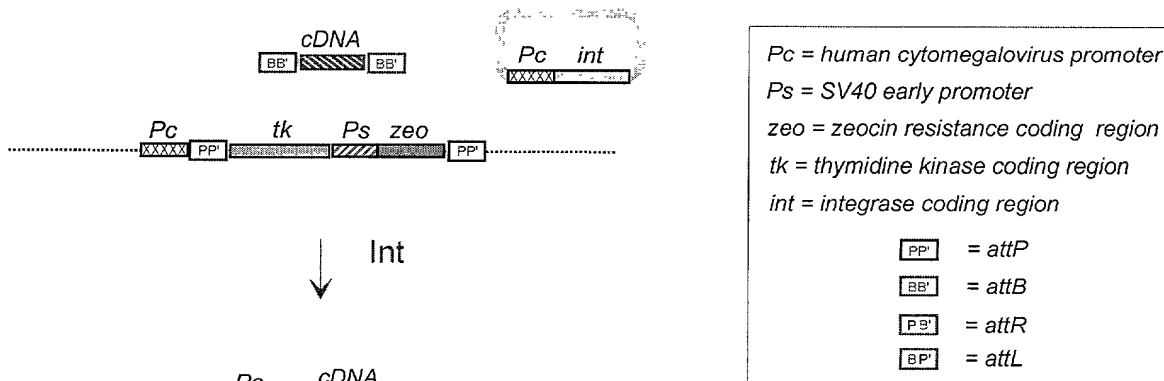## cDNA integration in mammalian cells
transient expression of *int*
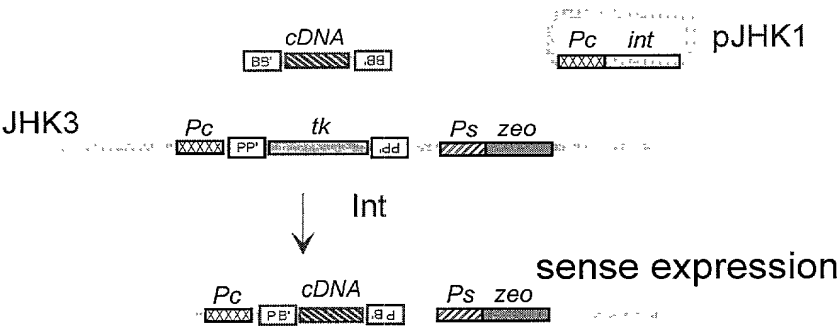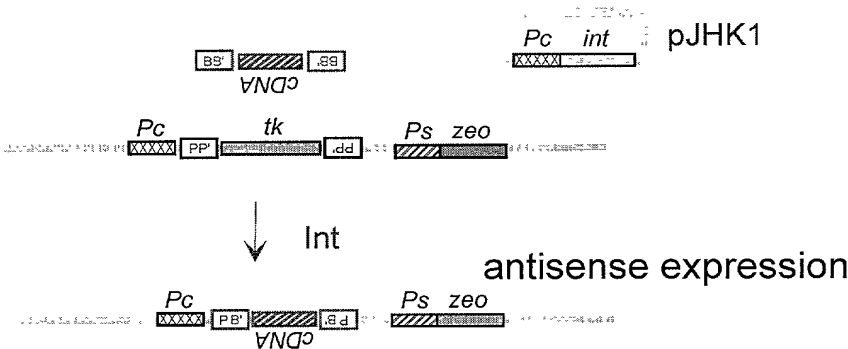
Strategy for cDNA integration in mammalian cells

A

cDNA
BB' [////] .BB

Pc int   pJHK1
XXXXX [....]

JHK3   Pc   tk   Ps   zeo
XXXXX PP' [========].dd   [////========]

↓ Int

Pc   cDNA   Ps   zeo   sense expression
XXXXX PB' [////] Pd   [////========]

B

BB' [////] .BB
cDNA (inverted)

Pc int   pJHK1
XXXXX [....]

Pc   tk   Ps   zeo
XXXXX PP' [========].dd   [////========]

↓ Int

Pc   PB' [////] Pd   Ps   zeo   antisense expression
XXXXX   [////========]
cDNA (inverted)

C

hpt
BB' [========] .BB   pJHK2
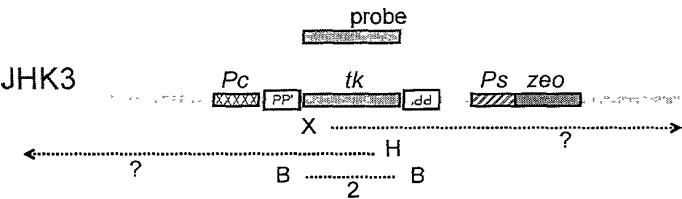
D

Pc   hpt   Ps   zeo   pcDNA3.1-hpt
XXXXX [====] [////====]

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin reistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

E   Single copy target construct in human cells

probe

JHK3   *Pc*   *tk*   *Ps*   *zeo*

X

H

B   B
2

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin reistance coding region
tk = thymidine kinase coding region

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL

F   PCR detection of DNA exchange

pJHK2

Pc   *int*
pJHK1

hpt
BB'   BB'

JHK3   *Pc*   *tk*   *Ps*   *zeo*

Int

Pc   *hpt*   *Ps*   *zeo*
PB'   PB'

1.2 kb
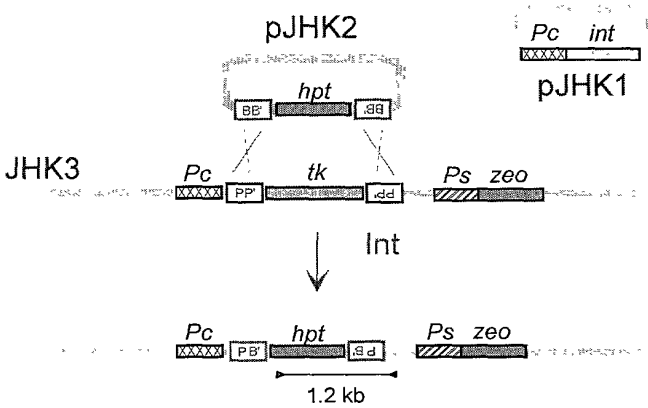
Figure 6

cDNA integration in plant cells
int expressed from target site

A

cDNA

BB' ▨▨▨ .BB

RB   *P*   *int*   *35S*   *codA*   *35S*   *npt*   *LB*
     PP'   PP'
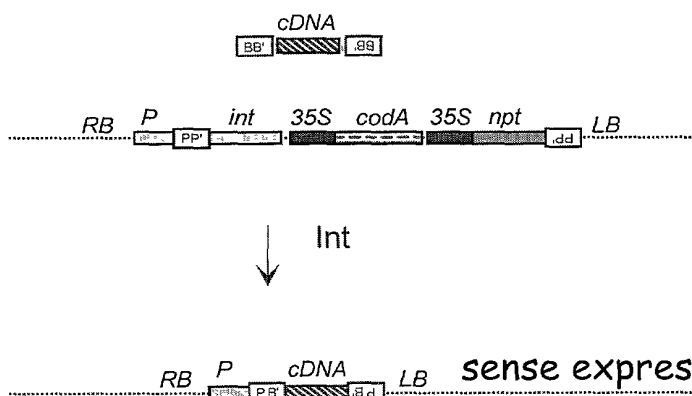
P = promoter	
35S = CaMV 35S promoter	
npt = kanamycin resistance coding region	
codA = cytosine deaminase coding region	
int = integrase coding region	
PP'	= *attP*
BB'	= *attB*
P B'	= *attR*
B P'	= *attL*

↓ Int

RB   *P*   cDNA   *LB*   **sense expression**
         P B' ▨▨▨ .B P

B

BB' ▨▨▨ .BB
cDNA

RB   *P*   *int*   *35S*   *codA*   *35S*   *npt*   *LB*
     PP'   PP'

↓ Int

RB   *P*   *LB*   **antisense expression**
     P B' ▨▨▨ P B
         cDNA
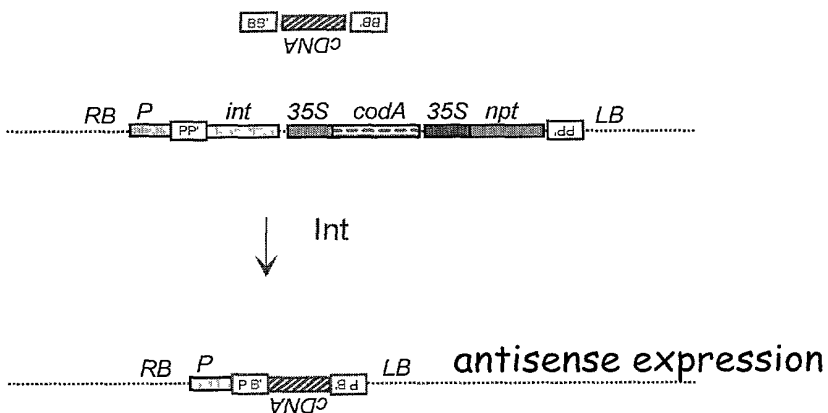
Figure 7

General strategy to incorporate only the trait gene
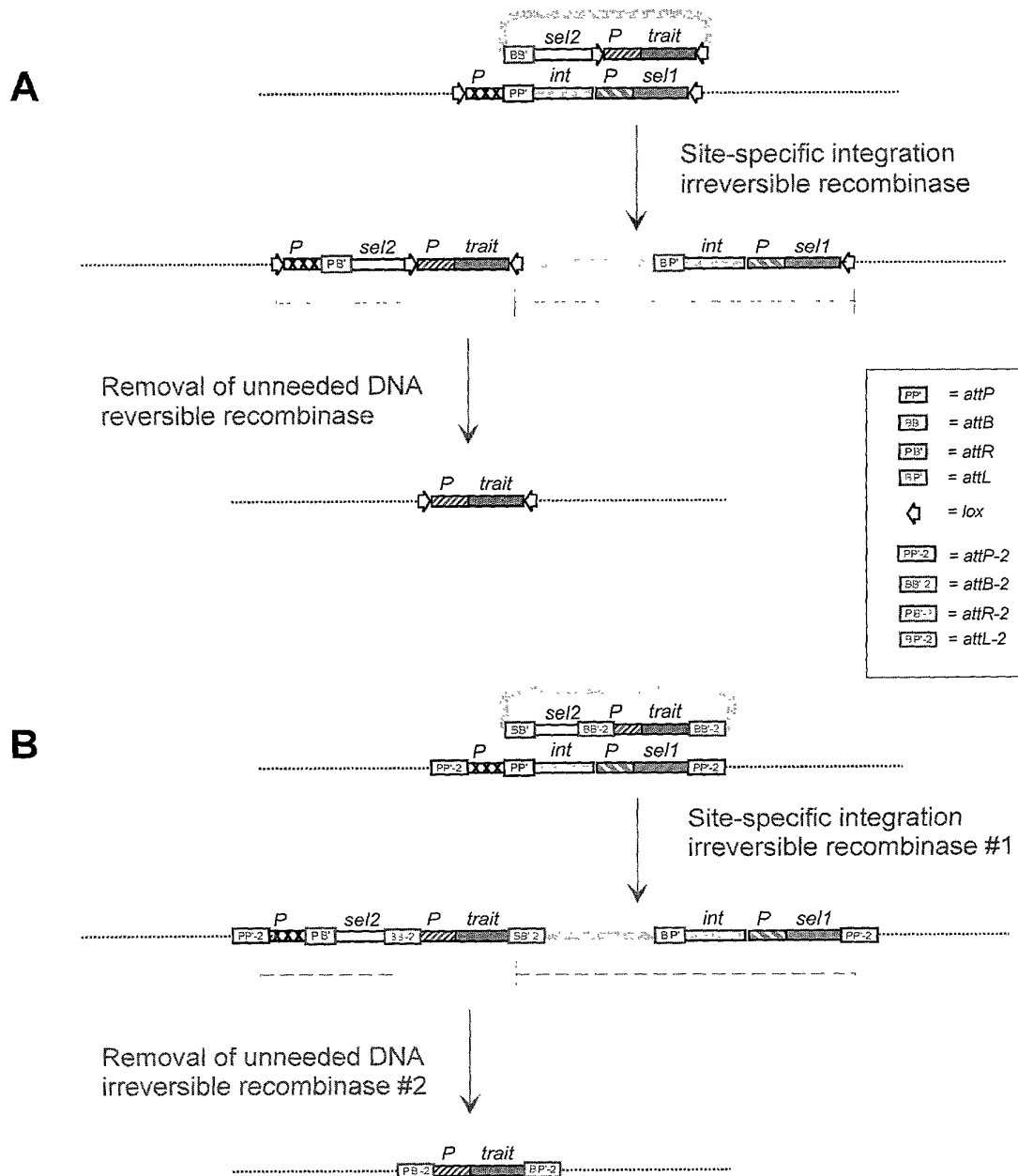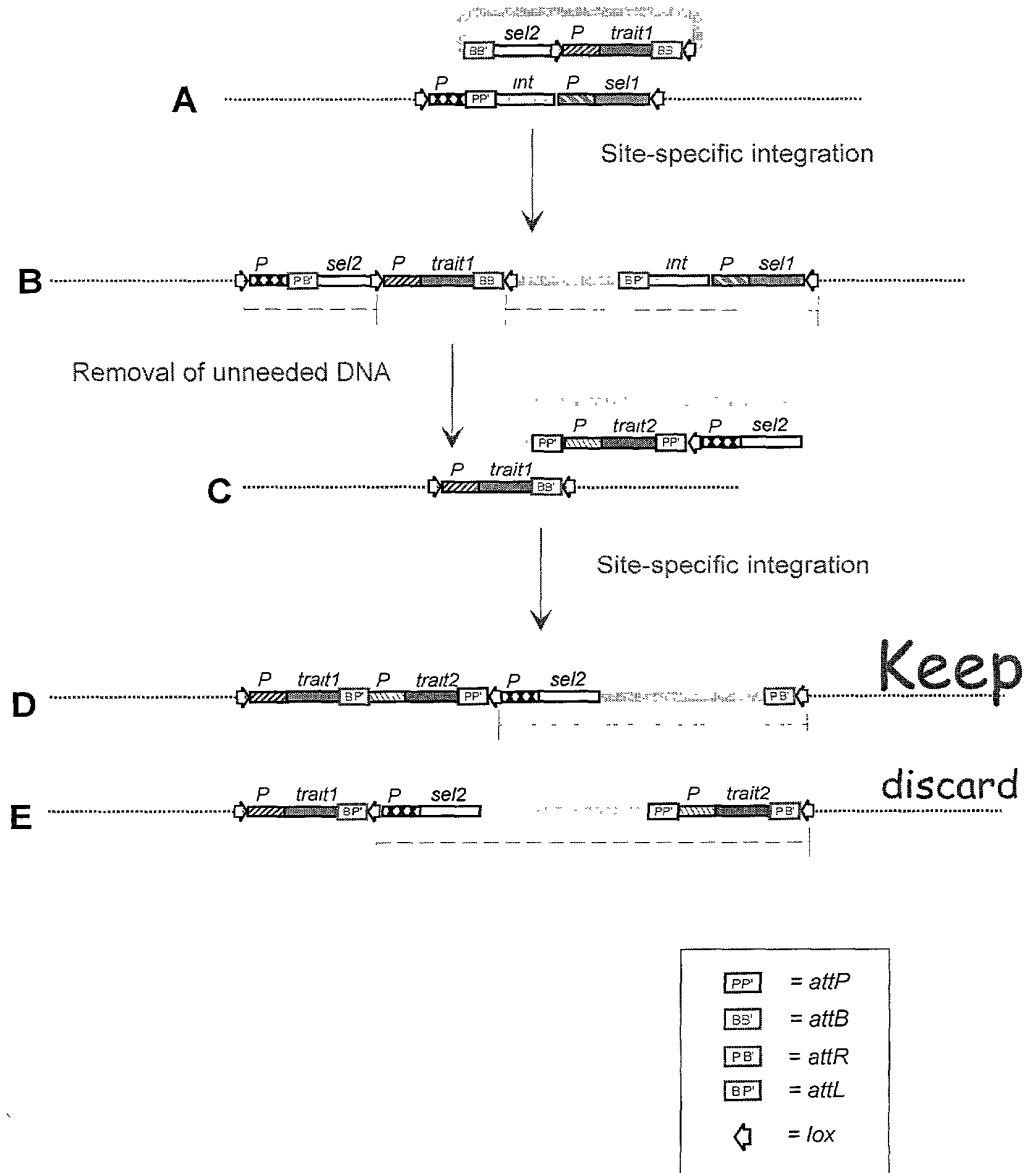
General strategy to stack genes, part1

Use of directly oriented sites

General strategy to stack genes, part2
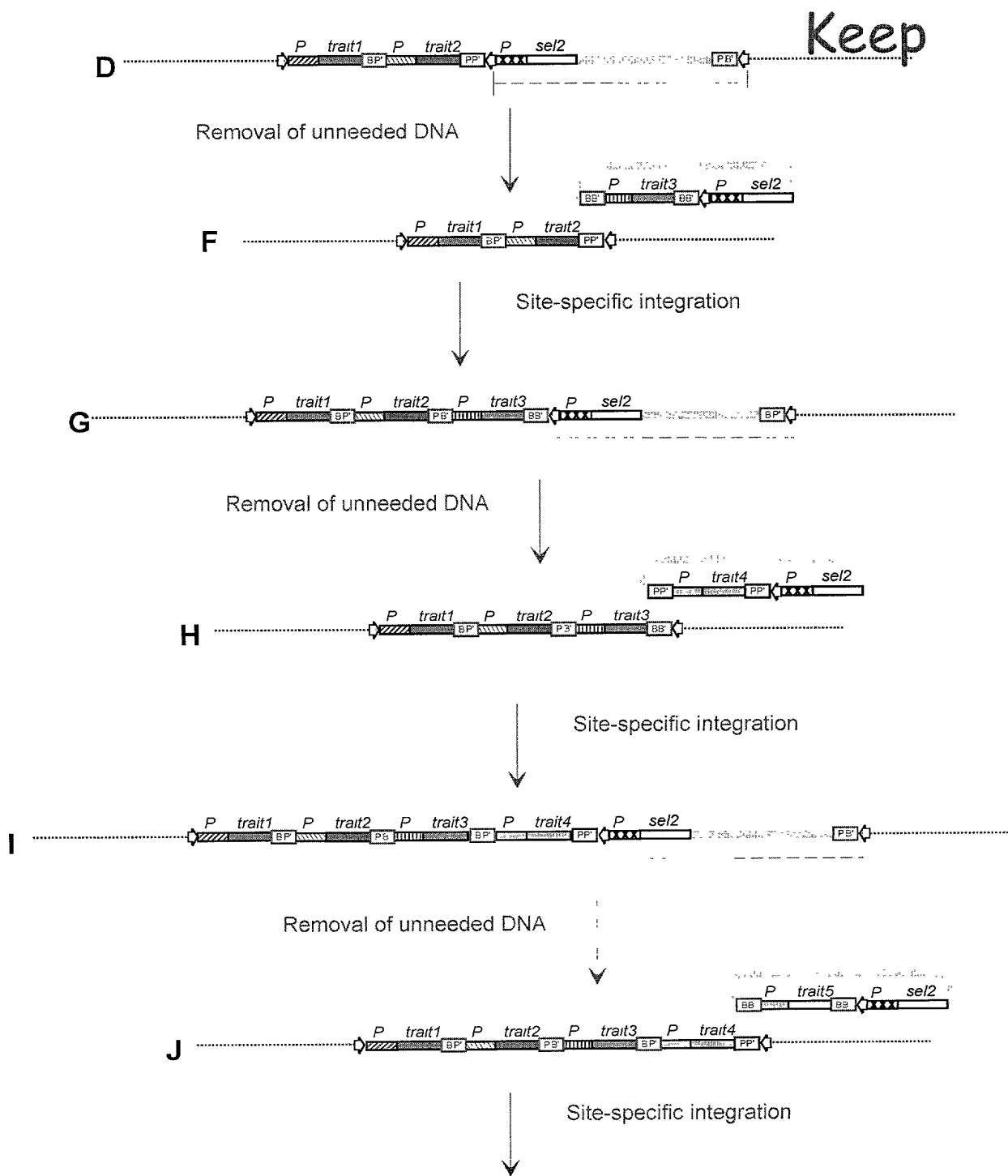
Use of directly oriented sites

Keep

D ·········· P trait1 P trait2 P sel2 ···· P B' ·········

Removal of unneeded DNA

P trait3 P sel2
B B' B B'

F ·········· P trait1 P trait2 ··········

Site-specific integration

G ·········· P trait1 P trait2 P trait3 P sel2 ···· B P' ··········

Removal of unneeded DNA

P trait4 P sel2
P P' P P'

H ·········· P trait1 P trait2 P trait3 ··········

Site-specific integration

I ·········· P trait1 P trait2 P trait3 P trait4 P sel2 ···· P B' ··········

Removal of unneeded DNA

P trait5 P sel2
B B B B

J ·········· P trait1 P trait2 P trait3 P trait4 ··········

Site-specific integration

General strategy to stack genes, part1

Use of inverted sites
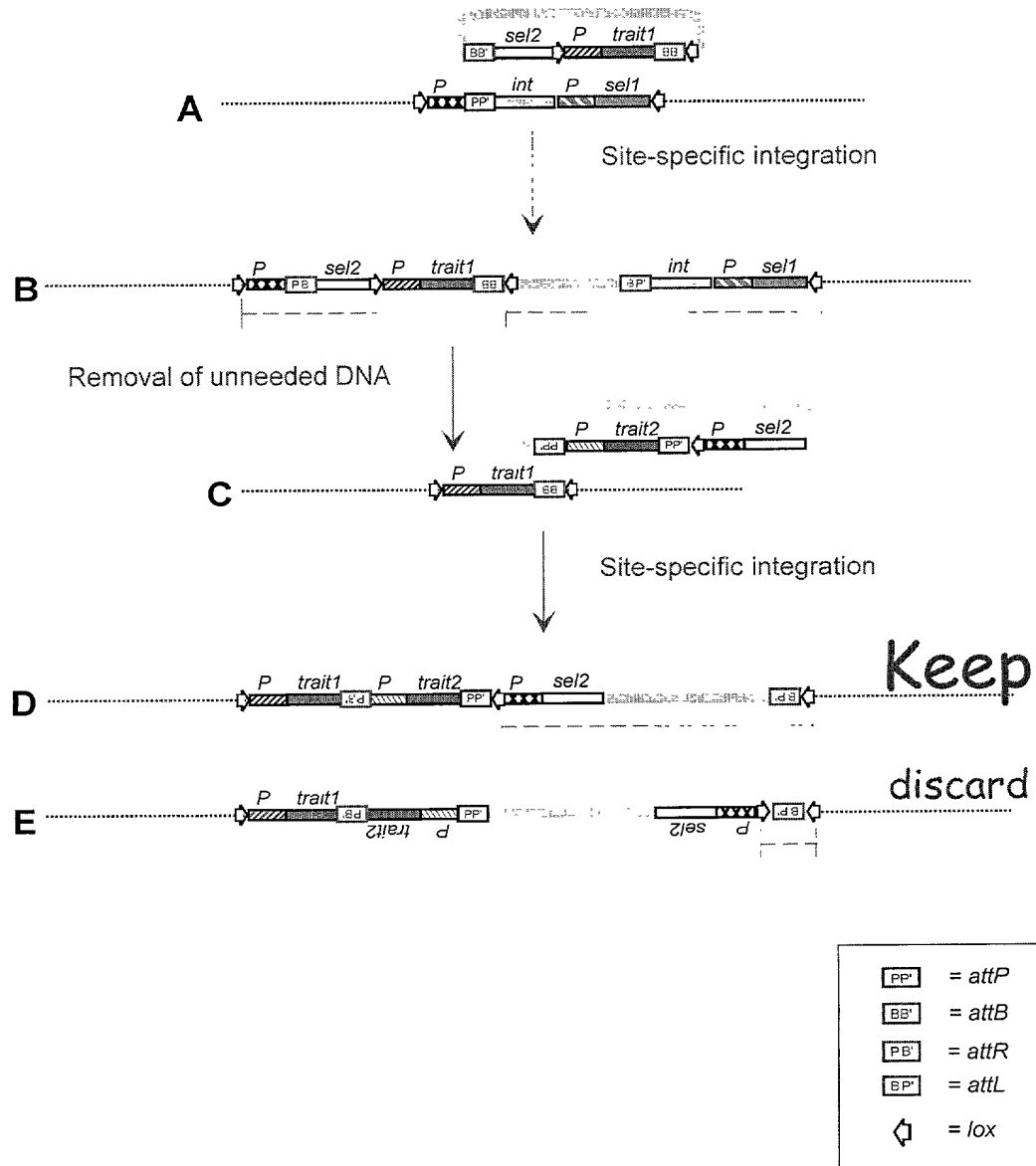
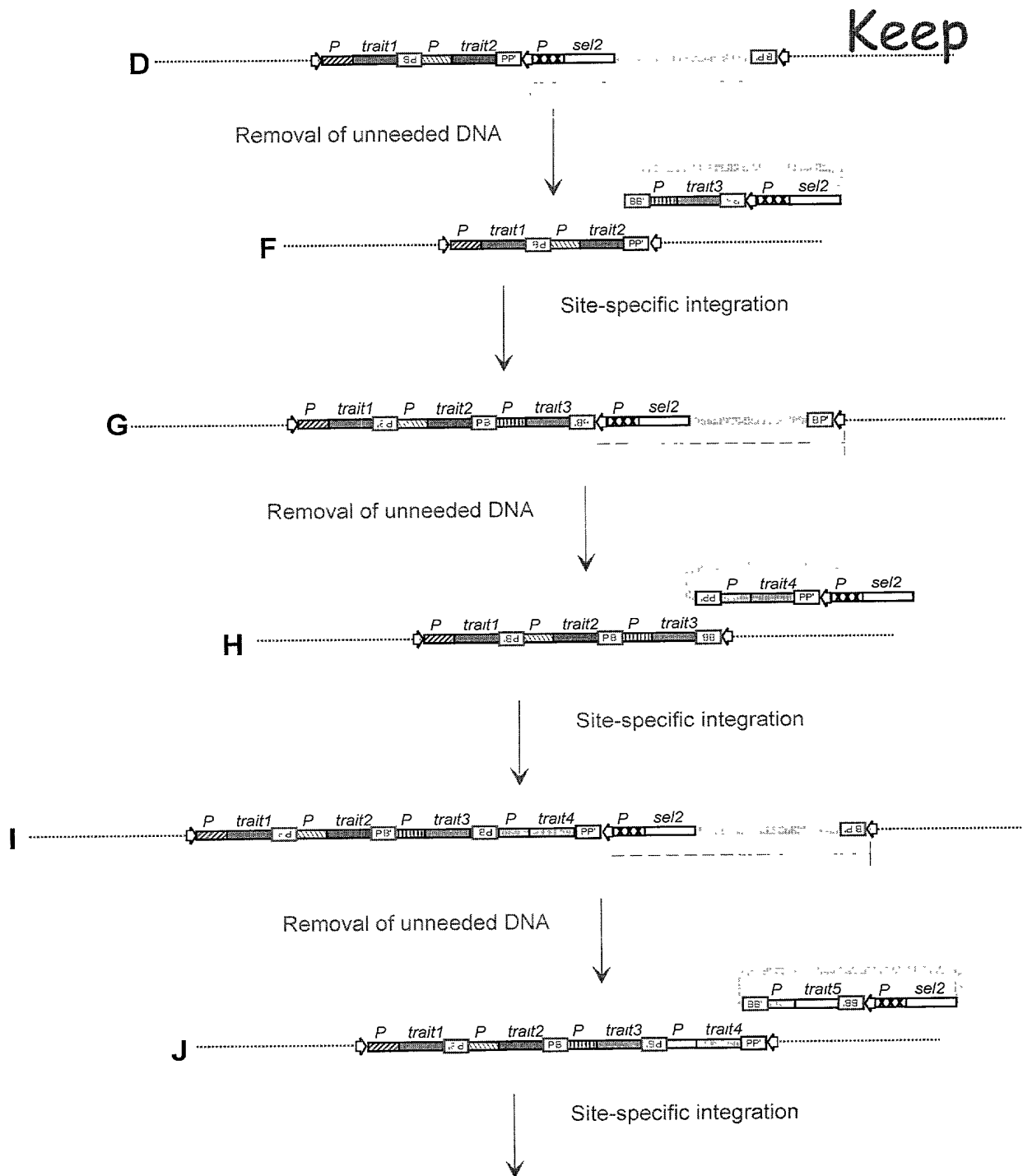General strategy to stack genes, part2

Use of inverted sites

Keep

D — P trait1 P trait2 P sel2 — PP' — BP

Removal of unneeded DNA

P trait3 P sel2
BB'

F — P trait1 P trait2 — PP'

Site-specific integration

G — P trait1 P trait2 P trait3 P sel2 — BP

Removal of unneeded DNA

P trait4 P sel2
dd — PP'

H — P trait1 P trait2 P trait3

Site-specific integration

I — P trait1 P trait2 P trait3 P trait4 P sel2 — dB

Removal of unneeded DNA

P trait5 P sel2
BB'

J — P trait1 P trait2 P trait3 P trait4 — PP'

Site-specific integration

Figure 10

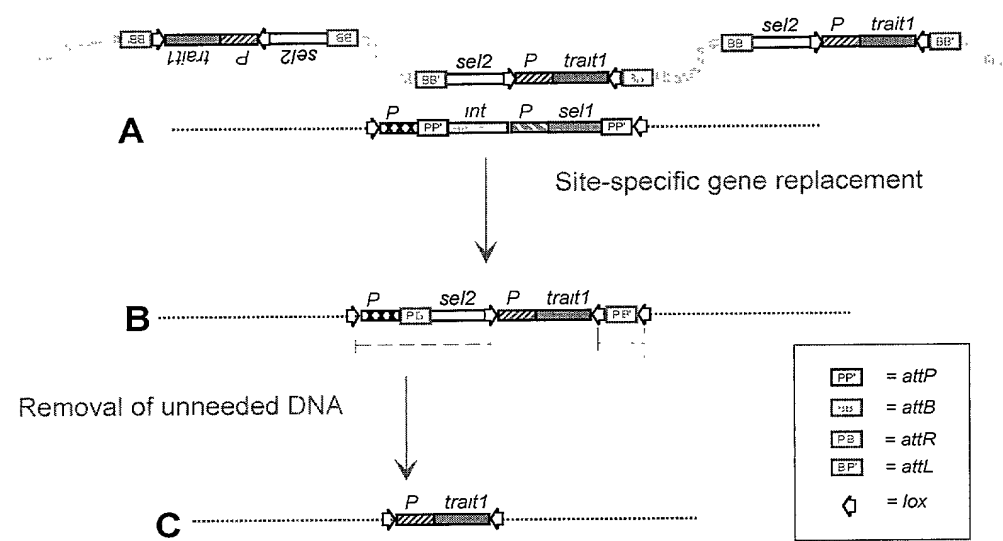Gene replacement in the host genome with directly oriented dual sites

Figure 11

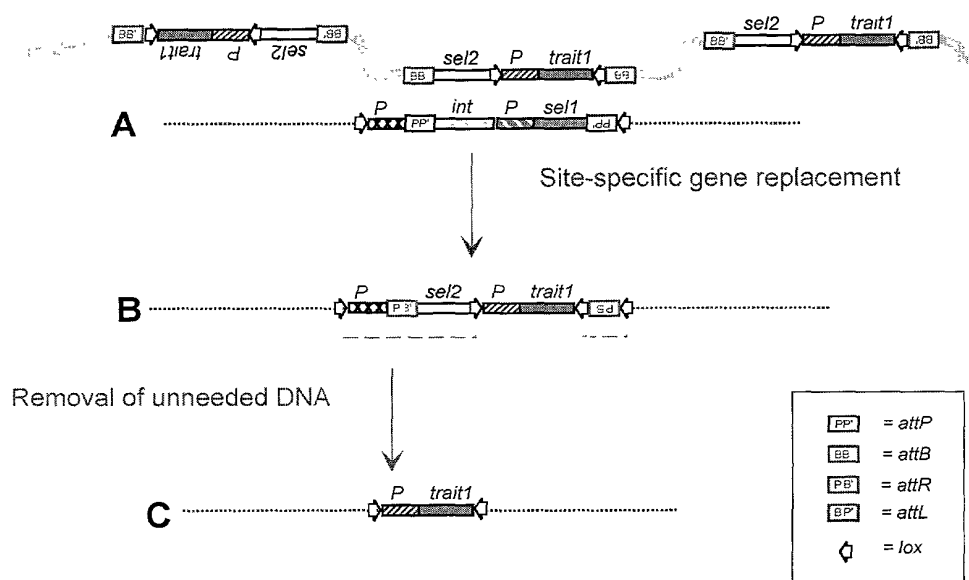Gene replacement in the host genome with inverted dual sites

Figure 12
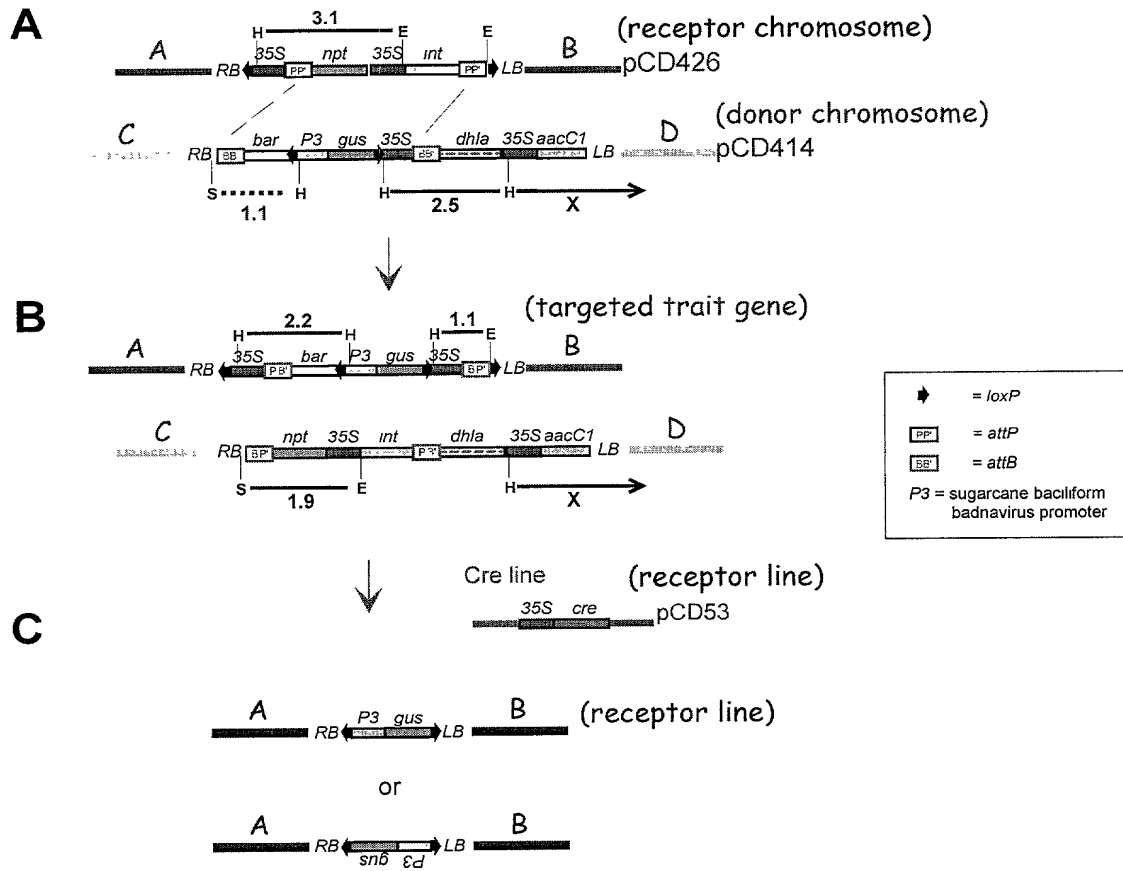
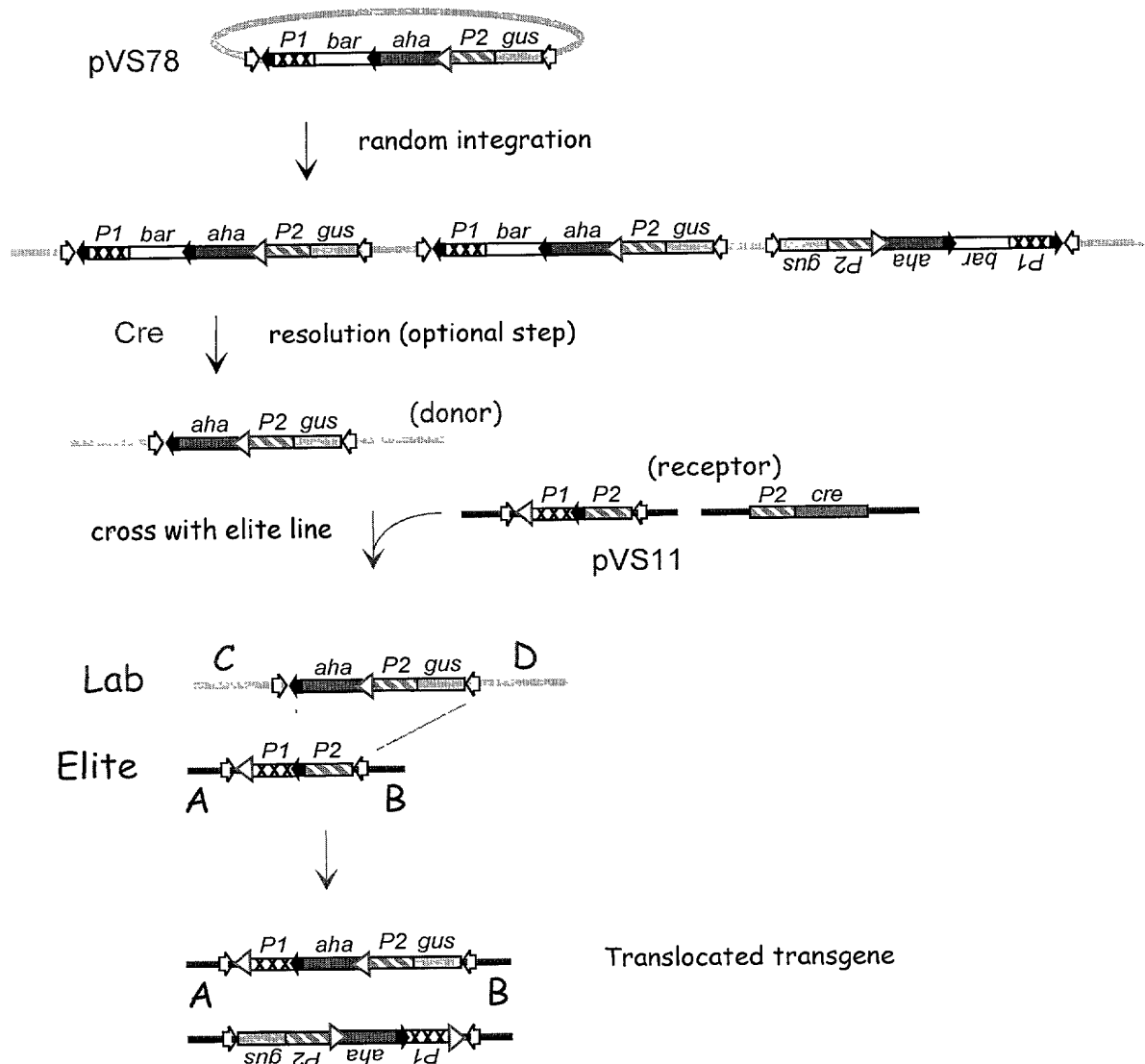Transgene translocation from one chromosome to another

Figure 13

Transgene translocation using reversible recombination systems

P1 = Rice Actin promoter

P2 = Maize Ubiquitin promoter

◁ = FRT

◀ = loxP   ◁ = lox511